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Relationship between changes in peroxide value and conjugated dienes during oxidation of sunflower oils with different degree of unsaturation

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RESUMEN

Relaciones entre los cambios en el índice de peróxidos e índice de dienos conjugados durante la oxidación de aceites de girasol con diferente grado de instauración.

El índice de peróxidos y la medida de dienos conjugados mediante la extinción específica a 232 nm se utilizan alternativamente para la evaluación de compuestos primarios de oxidación en estudios de oxidación de aceites y grasas. El objetivo de este estudio fue definir las relaciones entre ambas medidas en aceites de girasol con diferente grado de insaturación. Los aceites estudiados fueron aceite de girasol convencional (SO) y aceite de girasol alto oleico (HOSO). Con fines comparativos, también se estudió el linoleato de metilo, cuyos hidroperóxidos presentan estructura de dienos conjugados. Las muestras fueron oxidadas a 40 °C en la oscuridad. Con el fin de acelerar la oxidación a 40 °C, se eliminaron los antioxidantes naturales de los aceites, y se aplicó una relación superficie/volumen de aceite elevada (0.8 cm^{-1}) para garantizar total disponibilidad de aire. Los resultados obtenidos se ajustaron a regresiones lineales con elevados coeficientes de correlación (0.9988, 0.9991 y 0.9977 para el linoleato de metilo, SO y HOSO, respectivamente). Sin embargo, se encontraron diferencias significativas entre las pendientes de las rectas (0.0974, 0.0854 y 0.0503 para el linoleato de metilo, SO y HOSO, respectivamente), lo que indica la formación en los aceites de hidroperóxidos no conjugados formados a partir del ácido oleico, incluso en el aceite SO, de alto grado de insaturación. En consecuencia, sólo el índice de peróxidos sería un índice de confianza para la evaluación de los hidroperóxidos en estudios de oxidación con aceites de diferente grado de insaturación, salvo que se utilicen rectas de calibración adecuadas.

PALABRAS-CLAVE: *Ácidos grasos – Dienos conjugados – Hidroperóxidos – Índice de peróxidos – Linoleato de metilo – Oxidación*.

SUMMARY

Relationship between changes in peroxide value and conjugated dienes during oxidation of sunflower oils with different degree of unsaturation.

Peroxide value and the determination of conjugated dienes by UV absorption at 232 nm are normally applied alternatively for the evaluation of primary oxidation compounds. The objective of this study was to define the relationship between both indexes during oil oxidation in sunflower oils with different degree of unsaturation. The oils studied were conventional sunflower oil (SO) and high oleic sunflower oil (HOSO). For comparative purposes, methyl linoleate, whose hydroperoxides are conjugated diene hydroperoxides, was also studied. Samples were oxidized at 40 °C in the dark. In order to accelerate oxidation at 40 °C, the natural antioxidants present in the oils were removed, and a high surface to oil volume ratio (0.8 cm^{-1}) was applied to guarantee total air availability. Linear regressions with high correlation coefficients between peroxide values and conjugated dienes were found for the three lipid systems studied (0.9988, 0.9991 and 0.9977 for methyl linoleate, SO and HOSO, respectively). Significant differences in the slopes of the lines were found (0.0974, 0.0854 and 0.0503 for methyl linoleate, SO and HOSO, respectively), indicating the formation of non-conjugated hydroperoxides from oleic acid in the oils, even in the oxidation of SO with a high degree of unsaturation. Consequently, only peroxide value would be reliable for the evaluation of primary oxidation compounds in oils of different degree of unsaturation, unless adequate calibration lines (peroxide value *versus* conjugated dienes) are applied.

KEY-WORDS: Conjugated dienes – Fatty acids – Hydroperoxides – Methyl linoleate – Oxidation – Peroxide value.

1. INTRODUCTION

Lipid oxidation takes place through a set of autocatalytic reactions that produce a high number of new compounds. Hydroperoxides, the primary oxidation compounds, decompose giving rise to a variety of secondary oxidation products. Thus, the difficult evaluation of the oxidation status in oils requires the application of more than one analytical method for a better understanding of the level of both primary and secondary oxidation compounds present in a certain sample (Frankel, 2005).

There are thousands of papers on the evolution of oil oxidation where different analytical methods are applied. In some studies, the results are not easily interpreted due to the varying significance of the methods applied (peroxide value, conjugated dienes, anisidine value, level of pentane or hexanal, TBARS, UV extinction at 270 nm, etc.), and to the unspecified conditions of oxidation storage, in particular, the surface to oil volume ratio, which influences the availability of air.

The analysis of primary oxidation compounds is always carried out either by the peroxide value, normally determined by titration of the iodine released from the reaction of peroxides with potassium iodide, or, alternatively, by the determination of specific extinction at 232 nm, since the hydroperoxides of polyunsaturated fatty acids, the most susceptible fatty

acids to undergo oxidation, have a strong absorbance at 232 nm. The latter is especially useful for small samples and has the advantage of being non-destructive (Dobarganes and Velasco, 2002).

In this context, the main objective of this study was to define the relationship between peroxide value and conjugated dienes in sunflower oils with different degree of unsaturation. The oils studied were conventional sunflower oil (SO) and high oleic sunflower oil (HOSO). For comparative purposes, methyl linoleate, whose hydroperoxides are conjugated diene hydroperoxides, was also studied. Samples were oxidized at 40 °C in the dark. In order to accelerate oxidation at 40 °C, the natural antioxidants present in the oils were removed, and a high surface to oil volume ratio (0.8 cm^{-1}) was applied to guarantee total air availability. The samples were kept in the dark to prevent photoxidation, since some hydroperoxides would not be conjugated. Results in oils were compared to those obtained for methyl linoleate, from which conjugated diene hydroperoxides form as the only primary oxidation products.

2. MATERIALS AND METHODS

2.1. Samples

Standard methyl linoleate was purchased from Nu-Chek-Prep (Elysian, MN, USA). Conventional sunflower oil (SO) and genetically modified high-oleic sunflower oil (HOSO) were supplied by Koipe S.A. (Andújar, Jaén, España).

2.2. Sample treatments

Aluminum oxide treatment

SO and HOSO were treated to remove naturally occurring tocopherols. The procedure applied was that described by Yoshida *et al*. (1992), which consists of passing 100 g of oil through a column with 200 g of activated aluminum oxide (Al_2O_3: aluminum oxide 90, standardized for column chromatographic adsorption analyses, Merck,). Aluminum oxide was activated in an oven at 200 °C for at least 4 hours. After this treatment, tocopherols were not detected in the samples.

Storage conditions

Two identical independent samples (10 g) of methyl linoleate and 30 g of SO and HOSO were placed in open beakers (surface to oil volume ratio was 0.8 cm^{-1}) in a temperature-controlled chamber at 40 °C in the dark. Samples were taken periodically until an increase in polymeric compounds, corresponding to advanced oxidation, was observed (Márquez-Ruiz *et al*., 1996). In the case of the methyl linoleate, the samples were stored with a difference of 12 hours to take out samples at adequate oxidation periods (see table 1).

2.3. Analytical determinations

Peroxide value

Peroxide value (PV) was determined by the iodometric assay according to IUPAC standard method 2.501(IUPAC, 1992).

Conjugated dienes

Spectrophotometric determination of conjugated dienes by UV absorption at 232 nm was carried out according to AOCS standard method 2.501 (AOCS, 1998).

Fatty acid composition

Fatty acid composition was determined by GC once derived to fatty acid methyl esters with 2N KOH in methanol at room temperature, according to IUPAC Standard Method 2.301 (IUPAC, 1992).

Analysis of polymers

Polymers were determined by high performance size exclusion chromatography (HPSEC) following

Table 1
Evolution of oxidation at 40 °C in two independent samples of methyl linoleate

Sample 1			Sample 2		
Oxidation time (h)	Peroxide value (meq O_2/kg)	K 232	Oxidation time(h)	Peroxide value (meq O_2/kg)	K 232
0	2.1	1.72	0	2.1	1.72
6	8.2	2.16	12	21.2	3.07
9	22.6	3.76	15	38.0	4.42
12	52.8	5.96	18	66.0	6.60
24	171	18.02	21	84.5	8.61
27	203	20.63	36	250	25.20
30	255	25.12	39	291	30.42
33	299	29.17	42	353	33.32
48	545	54.67	45	406	38.80
51	633	64.07	–	–	–

IUPAC Standard Method 2.508 (IUPAC, 1992). Oil samples were diluted at 40-50 mg/mL in tetrahydrofuran and analyzed directly in an HPSEC chromatograph. The chromatograph was equipped with a Rheodyne 7725i injector with a 10-µL sample loop, a Waters 510 HPLC pump (Waters Associates, Milford, MA, USA), two 100 and 500 Å Ultrastyragel columns connected in series (Agilent Technologies, Milford, MA, USA), 25 cm x 0.77 cm I.D., packed with a porous, highly cross-linked styrenedivinylbencene copolymer (< 5µm), and a refractive index detector (Agilent Technologies, CA, USA).

Statistical analysis

Statistical analysis was performed by Microsoft Excel 2000 (Microsoft Co., WA, and USA). Comparisons between means were made by the Student's *t* test. Significance was defined at $P < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the evolution of oxidation at 40 °C in two independent samples of methyl linoleate. It should be noted that the main objective of this study was to know the intrinsic relationship between two analytical determinations, which should not be affected by oxidation conditions. Thus, only one sample would be necessary in the experiments. Nevertheless, the use of two samples in methyl linoleate made it possible to check differences in oxidation under identical storage conditions. It can be observed that high PV were obtained after only 50 hours of storage under the conditions applied (40 °C, S/V = 0.8 cm^{-1}).

Figure 1 shows the curves corresponding to the PV evolution in the two independent samples of methyl linoleate, where differences can be observed since the first hours of storage. The only apparent difference in the oxidation conditions was the time for sampling, which should not influence the oxidation under conditions of high air availability. Thus, the role of uncontrolled variables should be emphasized, as in other studies carried out under different conditions (Rodrigues Machado *et al.*, 2007).

Figure 2 shows the relationships between PV and K232. As mentioned above the differences in the rate of oxidation did not influence the correlation between both sets of values. As can be observed, high and similar correlation coefficients were found between the results of both determinations. Furthermore, there were no significant differences either in the slope or in the intercept ($P < 0.0001$).

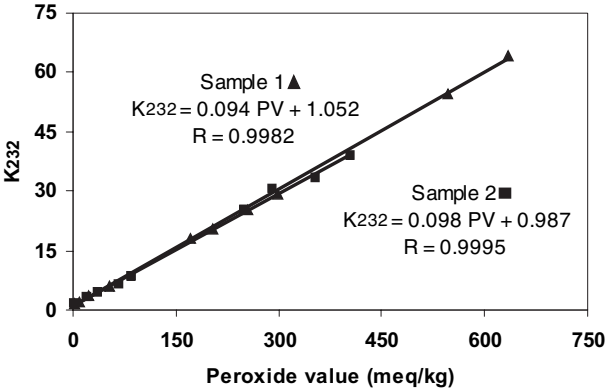

Figure 2.
Linear correlation between K232 and PV in two independent samples of methyl linoleate.

In the experiments designed for the oxidation of the oils, polymeric compounds were also measured. This analysis made it possible to decide the end of the assays, since it has been previously demonstrated that a significant increase in polymeric compounds is characteristic of the stage of advanced oxidation (Márquez-Ruiz *et al.*, 1996; Martín Polvillo *et al.*, 2004). Tables 2 and 3 show the results obtained for SO and HOSO, respectively; and Figure 3 shows a graphic illustration of the great differences attributed to the degree of unsaturation under identical conditions and sampling times. In the last column of the two tables the slow increase of polymeric compounds can be seen. The major compounds formed are hydroperoxides and it is not until the rapid decomposition of hydroperoxides when polymers become quantitatively important (Márquez-Ruiz *et al.*, 1996).

Figure 4 shows the relationship between the increase in PV and K232 for both sunflower oils and includes the regression lines and correlation coefficients found. The values for the regression

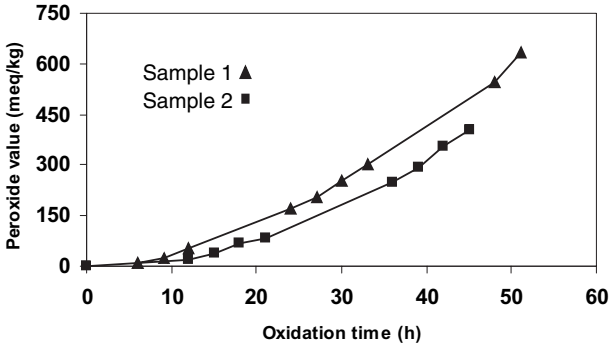

Figure 1.
Evolution of oxidation in two independent samples of methyl linoleate.

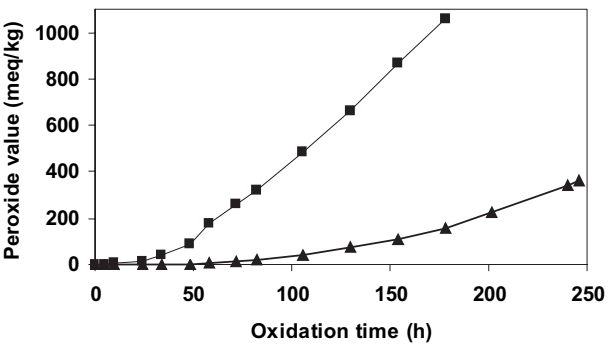

Figure 3
Evolution of oxidation in stripped-tocopherol high linoleic (■) and high oleic (▲) sunflower oils.

Table 2
Evolution of oxidation in conventional (high linoleic) sunflower oil

Oxidation time (h)	Peroxide value (meq O_2/kg)	K 232	Polymers (%)
0	0.5	2.53	<0.5
5	2	2.59	<0.5
10	5	2.56	<0.5
24	16	4.06	<0.5
34	41	6.06	<0.5
48	92	12.00	<0.5
58	176	17.97	0.7
72	262	26.09	1.0
82	321	28.69	1.2
106	486	42.19	2.0
130	661	60.32	3.1
154	865	79.14	4.3
178	1060	91.43	6.5

analysis were obtained from the difference between the values in tables 2 and 3 and those corresponding to the initial oils (oxidation time = 0). As can be observed, a lower slope was obtained for the oil of a lower degree of unsaturation, which could be explained by the differences in fatty acid composition. In this respect, in Table 4 the lines are compared with that obtained for methyl linoleate (samples 1 + sample 2). Standard errors and number of data are also given. Significant differences were found for the slopes ($P < 0.001$) although not for the intercepts. It can be seen that not only the maximum slope corresponded to methyl linoleate, but also significant differences were found between the three slopes as indicated in Table 4.

Figure 5 shows the structure of major hydroperoxides from oleic and linoleic acid, the two major unsaturated fatty acids in both sunflower oils. As can be observed, only hydroperoxides from linoleic acid contribute to the increase in the conjugated diene value. Thus, the increase in hydroperoxides from oleic acid would increase the PV but not the UV absorption at 232 nm. Consequently, if the significant differences between the three slopes were attributed to differences in fatty acid composition, this would mean that even in SO a significant amount of monounsaturated fatty acids is oxidized under the conditions applied.

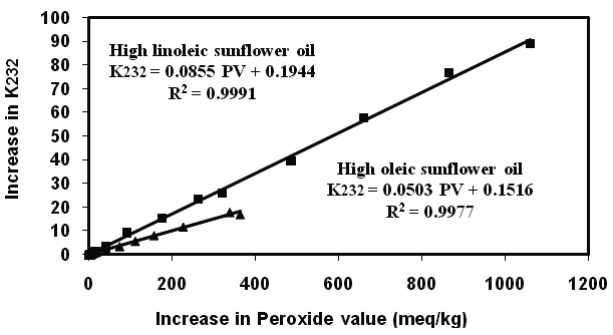

Figure 4.
Linear correlation between K232 and PV in stripped-tocopherol high linoleic (■) and high oleic (▲) sunflower oils.

Table 3
Evolution of oxidation in high oleic sunflower oil

Oxidation time (h)	Peroxide value (meq O_2/kg)	K 232	Polymers (%)
0	0.5	1.61	n.d.
5	0.6	1.66	n.d.
10	0.5	1.73	n.d.
24	1.6	1.71	n.d.
34	2.1	1.67	n.d.
48	3.9	1.97	<0.5
58	8.3	2.06	<0.5
72	12.8	2.47	<0.5
82	18.4	2.84	<0.5
106	42.2	3.94	<0.5
130	74.1	5.30	<0.5
154	112	7.49	<0.5
178	157	9.89	<0.5
202	227	13.531	0.7
240	339	19.71	1.3
246	364	18.84	1.7

N.d., not detected

Table 5 shows the quantitative determination of fatty acids for selected samples. The quantitative data in the table (g/100 g oil) has been calculated from the fatty acid composition (% on total fatty acids) by assuming that the major saturated fatty acid (C 16:0) does not undergo degradation and consequently its percentage remains constant as it is independent of the sample composition (Dobarganes and Pérez-Camino, 1988). The addition of the quantified fatty acid constituted the remaining nonpolar FAME, and the difference from the initial FAME, assumed to be 100, are the oxidized FAME. This simple calculation facilitates the determination of the total loss of fatty acids during oxidation as well as the individual loss of each unsaturated fatty acid. Due to analytical errors, only the samples containing more than 3-4 % of oxidized fatty acids are useful to check the individual losses of fatty acids.

As can be observed, in high linoleic sunflower oil the oxidation took place mainly in the linoleyl group of the triacylglycerols, while the loss of oleyl group was minimum throughout the total oxidation period. However, in high oleic sunflower oil, due to the high relative content in oleic acid, the decrease of oleic acid was similar to that found for linoleic acid. For example, after 154 hours, the losses of fatty acids from the initial oil were 0.8 and 10.1 g/100g for oleic and linoleic acids, respectively, in SO, while the losses for HOSO were 0.8 and 0.8 g/100g.

The relative loss of both fatty acids explains that the value of the slope in table 4 for SO was only slightly lower than that corresponding to methyl linoleate, as well as the great differences found in the slopes for the two oils. Under these circumstances, only changes in peroxide value would be comparable in oxidation studies of oils with different degree of unsaturation. When the use of conjugated dienes is necessary due to the need for applying a non-destructive method or due to

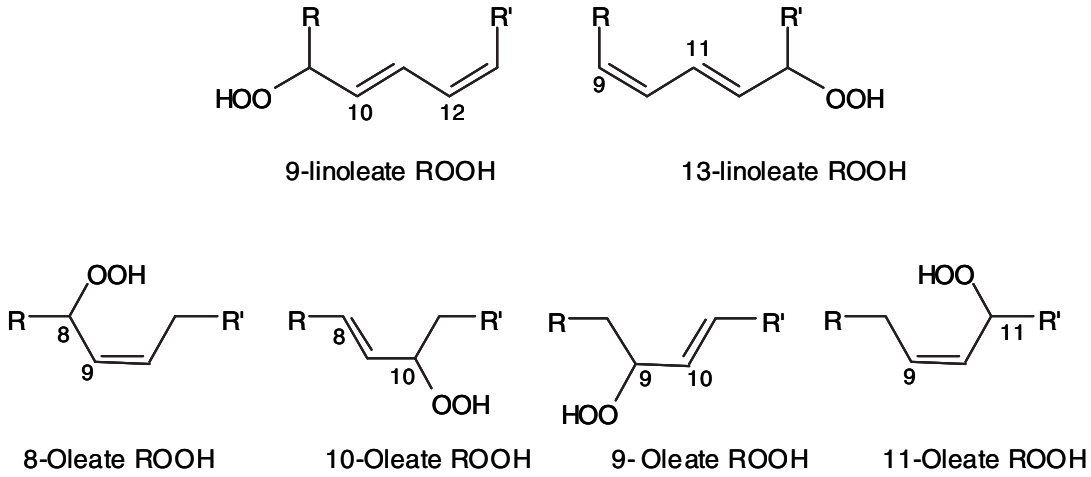

Figure 5.
Structures of major hydroperoxides coming from methyl linoleate and oleate.

limitations in the amount of sample, the use of the appropriate calibration lines between peroxide value and conjugated dienes would be useful to improve the determination of primary oxidation compounds.

4. CONCLUSIONS

1. An excellent linear correlation between peroxide value and conjugated dienes was found whatever the system studied.

2. The formation of conjugated dienes depended on the degree of unsaturation of the oil, since the presence of non-conjugated hydroperoxides from oleic acid is unavoidable. The relative amount of non-conjugated hydroperoxides is higher as the content of oleic acid increases in the oil.

3. When the analysis of conjugated dienes has to be applied for the indirect evaluation of hydroperoxides in oils and fats, the use of calibration lines (Peroxide Value *vs* conjugated dienes) for oils of different degree of unsaturation would improve the determination of primary oxidation compounds.

Table 4
Linear correlation analysis between K232 and PV in methyl linoleate

Sample	Slope	Intercept	Correlationcoefficient	n
Methyl linoleate	0.0974 ± 0.0011*[a]	0.70 ± 0.322[a]	0.9988	19
High linoleic sunflower oil	0.0854 ± 0.0011[b]	0.19 ± 0512[a]	0.9991	13
High oleic sunflower oil	0.0503 ± 0.0009[c]	0.15 ± 0.134[a]	0.9977	16

Linear equation K_{232} = A*PV + B; PV, Peroxide value; A, slope; B, intercept
* Mean value ± Standard error; Different superscript letters for each analysis indicate significant differences between samples.($p < 0.05$).

Table 5
Quantitative determination of fatty acid methyl esters (g/100g oil) in selected samples

Oil	Oxidation time (h)	C16:0	C18:0	C18:1	C18:2	Others	Nonpolar FAME	Oxidised FAME
High linoleic sunflower oil	0	6.4	4.4	26.5	61.2	1.5	100	0
	34	6.4	4.4	26.4	61.2	1.6	100	0
	48	6.4	4.4	26.4	60.4	1.5	99.0	1.0
	72	6.4	4.4	26.5	59.0	1.8	98.0	2.0
	106	6.4	4.4	26.1	55.7	2.0	94.6	5.4
	154	6.4	4.3	25.6	50.1	1.9	88.3	11.7
High oleic sunflower oil	0	3.9	2.7	80.6	10.6	2.2	100	0.0
	48	3.9	2.7	80.5	10.5	2.0	99.6	0.4
	130	3.9	2.7	80.3	10.2	1.8	98.9	1.1
	154	3.9	2.7	79.8	9.8	1.8	98.0	2.0
	202	3.9	2.8	78.5	8.7	1.9	95.8	4.2
	246	3.9	2.7	78.2	7.9	2.6	95.3	4.7

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REFERENCES

AOCS. 1998. *Official methods and recommended practices of the American Oil Chemists' Society* (5th edition). Champaign, Illinois: American Oil Chemists' Society, ed. D. Firestone.

Dobarganes MC, Pérez-Camino MC. 1988. Fatty acid composition: a useful tool for the determination of alteration level in heated fats. *Rev. Franc. Corps Gras* **35**, 67-70.

Dobarganes MC, Velasco J. 2002. Analysis of Lipid Hydroperoxides.- *Eur. J. Lipid Sci. Technol.* **104,** 420-428.

Frankel E N. 2005. Methods to determine the extent of oxidation. In *Lipid Oxidation*, 2nd Edition, The Oily Press, England, 99-127.

IUPAC.1992. *Standard methods for the analysis of oils fats and derivatives*, 1st supplement to 7th edition. International Union of Pure and Applied Chemistry. Pergamon Press, Oxford.

Márquez-Ruiz G, Martín-Polvillo M, Dobarganes MC. 1996. Quantitation of oxidized triglyceride monomers and dimers as an useful measurement for early and advanced stages of oxidation, *Grasas y Aceites,* **47,** 48-53.

Martín-Polvillo M. Márquez-Ruiz G, Dobarganes M C. 2004. Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature. *J. Am. Oil Chem. Soc.* **81,** 577-583.

Rodrigues Machado E, Marmesat S, Abrantes S, Dobarganes M C. 2007. Uncontrolled variables in frying studies: differences in repeatability between thermoxidation and frying experiments. *Grasas y Aceites* **58**, 283-288.

Yoshida H, Kondo I, Kajimoto G. 1992. Participation of free fatty acids in the oxidation of purified soybean oil during microwave heating. *J. Am. Oil Chem. Soc.*, **69**, 1136-1140.

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